Pralatrexate with Vitamin Supplementation in Patients with Previously Treated, Advanced Non-small Cell Lung Cancer

Safety and Efficacy in a Phase 1 Trial

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Introduction: Pralatrexate is an antifolate designed for preferential tumor cell uptake and accumulation and received accelerated Food and Drug Administration approval in relapsed/refractory peripheral T-cell lymphoma. Pralatrexate 135 to 150 mg/m^2 every 2 weeks without vitamin supplementation was active in non-small cell lung cancer (NSCLC) although mucositis was dose limiting. This phase 1 study evaluated the safety of higher pralatrexate doses with vitamin supplementation to minimize toxicities.

Methods: Patients with stage IIIB/IV NSCLC received pralatrexate 150 to 325 mg/m^2 every 2 weeks with folic acid and vitamin B_{12} supplementation. Outcomes measured included adverse events (AEs), pharmacokinetics, and radiologic response.

Results: Thirty-nine patients were treated for a median of two cycles (range 1–16+). Common treatment-related grade 3 and 4 AEs by dose (≤190 mg/m^2 and >190 mg/m^2) included mucositis (33 and 40%) and fatigue (11 and 17%). Treatment-related serious AE (SAE) rates for doses ≤190 and >190 mg/m^2 were 0 and 20%, respectively. The response rate was 10% (95% confidence interval: 1–20%), including two patients with complete response (26+ and 32+ months) and two with partial response. Serum pralatrexate concentrations increased dose dependently up to 230 mg/m^2.

Conclusions: Pralatrexate with vitamin supplementation was safely administered to patients with previously treated NSCLC, and durable responses were observed. The recommended starting dose for phase 2 is 190 mg/m^2. A similar safety profile was observed in patients treated at 230 mg/m^2, although a higher serious AE rate was evident. Mucositis remains the dose-limiting toxicity of pralatrexate, and this study failed to demonstrate that vitamin supplementation prevents mucositis and failed to identify clinical predictors of mucositis. Individualized dose-modification strategies and prospective mucositis management will be necessary in future trials.

Key Words: Pralatrexate, Antifolate, Non-small cell lung cancer, NSCLC, Dose-finding.

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Pralatrexate (FOLOTYN Allos Therapeutics, Inc., Westminster, CO) is an antifolate-targeting dihydrofolate reductase (DHFR) and has a high affinity for the reduced folate carrier (RFC)-1.[1] RFC-1 is highly expressed on malignant tissues and it regulates the internalization of natural folates required for purine and pyrimidine biosynthesis.[2] In lymphoma cell lines, RFC-1 gene expression predicted the antitumor activity of pralatrexate,[3] and it is hypothesized that the high affinity of pralatrexate for RFC-1 leads to selective tumor cell accumulation. Pralatrexate is an efficient substrate for polyglutamylation by the enzyme folylpolyglutamyl synthetase (FPGS), with activity at FPGS greater than that of other antifolates (methotrexate, edatrexate, and aminopterin).[1] The increased uptake and increased polyglutamylation associated with pralatrexate have been shown to correlate with increased tumor growth inhibition in non-small cell lung cancer (NSCLC) xenograft models.[4]

Pralatrexate recently received accelerated approval by the U.S. Food and Drug Administration for the treatment of relapsed or refractory peripheral T-cell lymphoma based on the results of the PROPEL study.[5] Pralatrexate has also been studied in other types of lymphomas and solid tumors.[6] In particular, several studies have evaluated pralatrexate in patients with NSCLC as either monotherapy,[7,8] or in combination with taxanes.[9] Pralatrexate monotherapy was active at doses of 135 to 150 mg/m^2 every 2 weeks (q2w).[7,8] Mucositis was a common dose-limiting toxicity (DLT), but early monotherapy studies did not include administration of vitamin B_{12}

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and folic acid. In the phase 1 study of pralatrexate in combination with a taxane, mucositis was the most common DLT. Supplementation of vitamin B_{12} and folic acid in some patients reduced the incidence of mucositis and allowed the use of increased doses of pralatrexate.[9] In addition, data from studies of the antifolate pemetrexed (ALIMTA) have led to the routine administration of vitamin supplementation with this agent.[10] The objective of this phase 1 study (PDX-007) was to evaluate the safety, tolerability, pharmacokinetic (PK) profile, and optimal dose of pralatrexate with routine vitamin B_{12} and folic acid supplementation in patients with advanced, previously treated NSCLC.

PATIENTS AND METHODS

Study Design

This was a phase 1, nonrandomized, multicenter, dose-escalation study. Study participants were 18 years or older and had stage IIIB or IV NSCLC that was not potentially curable by standard chemotherapy, radiotherapy, or surgical procedures. Other inclusion criteria were at least one prior chemotherapy regimen, Karnofsky Performance Status $\leq$70%, life expectancy more than 3 months, and adequate hematologic, hepatic, and renal function. Key exclusion criteria were previous exposure to pralatrexate, diagnosis of another active concurrent malignancy, clinically significant pleural effusion or ascites, grade 3 or 4 edema, prior pneumonectomy, recent (within 2 weeks of enrollment) radiation therapy or chemotherapy (erlotinib or gefitinib within 1 week before enrollment), symptomatic central nervous system metastases, and other serious medical conditions. The study was conducted in accordance with the principles contained in the Declaration of Helsinki and was approved by the Institutional Review Boards at participating centers. Each patient provided written informed consent to participate.

Treatment

Each patient received pralatrexate intravenously (IV) once q2w. Each cycle of therapy was 4 weeks and consisted of two doses of pralatrexate. Patients received vitamin supplementation starting at least 7 days (day 7) before the first dose of pralatrexate and continued until discontinuation of pralatrexate. Vitamin supplementation consisted of vitamin B_{12} (1 mg intramuscular every 8 to 10 weeks) and folic acid (1 mg orally once daily).

Patients were enrolled sequentially into two treatment groups during the study: treatment group A followed by group B. treatment group A determined the maximum tolerated dose (MTD) of pralatrexate with vitamins based on a modified, accelerated titration method for dose escalation.[11] Treatment group B was a dose de-escalation phase to determine the recommended phase 2 dose using stricter DLT criteria. This phase followed a standard 3 + 3 method of enrollment into each cohort.

The first dose cohort in treatment group A was 150 mg/m^2 q2w by IV bolus (3–5 minutes). If no DLT was observed in the first patient after one cycle of therapy (two doses over 4 weeks), the next patient was enrolled at the next dose level (40 mg/m^2 increments until 270 mg/m^2 and 20% increments thereafter). If a DLT was observed in the first patient, five additional patients were enrolled in the cohort. If only the first patient in the cohort had a DLT after expansion to six patients, dose escalation continued to the next level. If $\geq$2 patients had a DLT with cohort expansion, dose escalation was stopped, and the MTD was defined as the previously tolerated dose. After the MTD was identified, the MTD cohort was expanded to include 16 patients in total to gain additional safety and tolerability experience at that dose level.

Because several patients in the expanded MTD cohort had mucositis as DLT at 270mg/m^2, additional cohorts were enrolled into treatment group B to evaluate lower doses. In addition, $\geq$grade 2 mucositis was added to the DLT definition. Another investigation undertaken in treatment group B was to evaluate the PK and safety profile in patients who received a protracted infusion (more than 60 minutes) of pralatrexate. To further evaluate if the safety profile may be improved using a longer infusion, subsequent cohorts in treatment group B received pralatrexate 230 mg/m^2 q2w by IV bolus, prolonged infusions, and/or lower doses of pralatrexate (40 mg/m^2 reductions).

Treatment was delayed in patients with active mucositis. Those patients who experienced grade 3 mucositis restarted pralatrexate at a 40% reduced dose after mucositis resolved. There was no change in dose for other nonhematologic grade 0 to 2 adverse events (AEs). The dose was delayed for 2 weeks in patients with other grade 3 nonhematologic treatment-related AEs and restarted as follows: if the AE resolved to grades 0 and 1, treatment was restarted at the same dose; if resolved to grade 2, treatment was restarted with a 20% dose reduction; if grade 3 persisted, the next dose was omitted. Pralatrexate treatment was discontinued for any grade 4 treatment-related nonhematologic AE. For treatment-related hematologic AEs, pralatrexate treatment was delayed in patients with grade 2 neutropenia and grade 2 thrombocytopenia. Treatment was delayed and could be restarted at a 20% reduced dose in patients with grade 3 neutropenia with fever or grade 4 neutropenia upon recovery. Prophylactic use of hematopoietic growth factors during cycle 1 was not permitted.

Assessments

Investigators recorded each AE including its relationship to pralatrexate treatment and its severity according to the National Cancer Institute Common Terminology Criteria for Adverse Events scale, Version 3.0. Blood samples were tested for red blood cell (RBC) folate, homocysteine (HC), and methylmalonic acid (MMA) levels at enrollment, before the first dose of pralatrexate, and after cycle 1 as a measure of vitamin deficiency and to measure the impact of vitamin supplementation. Serial PK plasma and urine samples were collected for 72 hours after the first dose of pralatrexate. Additional PK plasma samples were collected for 20 minutes after the second dose. If specimens were available, formalin-fixed paraffin-embedded (FFPE) tumor tissue was collected as part of the treatment initiation visit. Available FFPE tissue was tested for components of folate metabolism (RFC-1, DHFR, FPGS, thymidylate synthase [TS], glycinamide ribonucleotide formyltransferase, and gamma-glutamyl hydro-

lase) to identify biomarkers that may correlate with drug activity. The relative gene expression of RFC-1, DHFR, FPGS, TS, glycinamide ribonucleotide formyltransferase, and gamma-glutamyl hydrolase was evaluated using a reverse transcription polymerase chain reaction assay using standard techniques (Response Genetics, Inc., Los Angeles, CA).

Investigators evaluated radiologic response using serial computed tomography scans and tumor measurements. Treatment response was categorized using RECIST.[12] Responders continued to be followed for duration of response after the safety analyses were completed.

Dose-Limiting Toxicity

The definition of DLT in group A included the following: (1) ≥grade 3 treatment-related hematologic toxicity, excluding anemia, lasting for ≥7 days or requiring treatment with filgrastim or pegfilgrastim; (2) ≥grade 3 neutropenia with fever; (3) ≥grade 3 treatment-related nonhematologic toxicity, excluding nausea and vomiting; and (4) dose delay of more than 2 weeks for treatment-related toxicity. In group B, ≥grade 2 mucositis was added as a DLT to determine the recommended phase 2 dose.

Statistical Analysis

Data were analyzed for all patients who were enrolled and received at least one dose of pralatrexate. Baseline values and outcomes were summarized by treatment cohort.

RESULTS

Study Population

The 39 patients who received at least one dose of pralatrexate comprised the efficacy and safety population. Baseline characteristics of these 39 patients are summarized in Table 1. Of these, 29 discontinued the study for progressive disease, 1 because of withdrawal of consent, 6 because of AEs, and 1 because of the development of an AE that resulted in more than one dose reduction of pralatrexate. At the time of reporting this article, two patients remained on study with sustained complete radiologic responses (26+ months and 32+ months).

The number of patients who received 150, 190, 230, 270, and 325 mg/m^2 was 1, 8, 11, 16, and 3, respectively, across both treatment groups. Of the 39 patients treated with pralatrexate, 29 received bolus injections over 3 to 5 minutes and 10 (5 each in the 190 and 230 mg/m^2 cohorts in treatment group B) received 1-hour infusions. Most patients (87%) were white and 59% were women. The mean age was 61 years. Although all histological subtypes were included in eligibility criteria, the majority (82%) were adenocarcinomas. Fifteen patients (38%) received one prior chemotherapy regimen, 7 (18%) received two prior, and 17 (44%) patients received three or more. The most commonly used prior chemotherapy regimens were platinum-based combinations (92%). Fourteen patients (36%) received prior pemetrexed, and prior treatment with epidermal growth factor receptor therapy (59%) was also permitted. Nineteen patients (49%) received prior radiation, four patients received surgical resection for early-stage or metastatic NSCLC before being enrolled on the study with progressive NSCLC.

Pralatrexate Dosing

Patients received pralatrexate for a median of two cycles (range 1–16+). The mean number of doses in the 150, 190, 230, 270, and 325 mg/m^2 cohorts was 4.0, 4.4, 6.7, 7.5,

TABLE 1. Baseline Patient Characteristics

Value	150 mg/m^2 (n = 1)	190 mg/m^2 (n = 8)	230 mg/m^2 (n = 11)	270 mg/m^2 (n = 16)	325 mg/m^2 (n = 3)	Total (n = 39)
Female, *n* (%)	1 (100)	4 (50)	5 (45)	12 (75)	1 (33)	23 (59)
Race, *n* (%)						
White	1 (100)	5 (63)	10 (91)	15 (94)	3 (100)	34 (87)
Black	0 (0)	3 (38)	1 (9)	1 (6)	0 (0)	5 (13)
Age (yr)						
≥65, *n* (%)	0 (0)	4 (50)	5 (45)	6 (38)	0 (0)	15 (38)
Mean (SD)	54.0 (—)	64.5 (10.4)	62.1 (10.5)	60.7 (7.9)	55.3 (7.1)	61.3 (9.1)
Adenocarcinoma	1 (100)	5 (63)	10 (91)	15 (94)	1 (33)	32 (82)
Squamous cell	0 (0)	2 (25)	0 (0)	0 (0)	1 (33)	3 (8)
Other histology	0 (0)	1 (12)	1 (9)	1 (6)	1 (33)	4 (10)
Prior therapy, *n* (%)						
Chemotherapy	1 (100)	8 (100)	11 (100)	16 (100)	3 (100)	39 (100)
Radiation	1 (100)	4 (50)	4 (36)	9 (56)	1 (33)	19 (49)
Resection	0 (0)	0 (0)	0 (0)	3 (19)	1 (33)	4 (10)
Other	0 (0)	3 (38)	7 (64)	11 (69)	1 (33)	22 (56)
No. of prior NSCLC chemotherapy regimens						
1	1 (100)	4 (50)	4 (36)	5 (31)	1 (33)	15 (38)
2	0 (0)	1 (13)	1 (9)	4 (25)	1 (33)	7 (18)
3+	0 (0)	3 (38)	6 (55)	7 (44)	1 (33)	17 (44)

NSCLC, non-small cell lung cancer.

TABLE 2. Dose Levels and Dose-Limiting Toxicity

Cohort	No. of Patients	Dose (mg/m^2)	Bolus (3–5 min) or Infusion (60 min)	Dose-Limiting Toxicity
A1	1	150	Bolus	—
A2	1	190	Bolus	—
A3	1	230	Bolus	—
A4	6	270	Bolus	1 Mucositis (Gr 3)
A5	3	325	Bolus	2 Mucositis (Gr 3)
A4 (expansion)	10	270	Bolus	2 Mucositis (Gr 3)
				1 Mucositis + fatigue (Gr 3 + 3)
				1 Mucositis + fatigue + headache (Gr 3 + 3 + 3)
B1	5	230	Bolus	3 Mucositis (Gr 2)
				1 Mucositis (Gr 2–3)
B2	5	230	Infusion	1 Mucositis (Gr 3)
				1 Mucositis + thrombocytopenia + neutropenia (Gr 2 + 3 + 3)
B3	2	190	Bolus	2 Mucositis (Gr 3)
B4	5	190	Infusion	2 Mucositis (Gr 2)

The maximum tolerated dose in part A was identified as 270 mg/m^2 because of dose-limiting toxicities in two of three patients at 325 mg/m^2; 10 additional patients were enrolled to the 270 mg/m^2 dose in the "expansion" cohort, and 4 of 10 had dose-limiting toxicities. Part B evaluated lower doses (230 and 190 mg/m^2) and extended 1-h infusions; grade 2 mucositis was added as a dose-limiting toxicity in part B only.

Gr, grade.

and 4.7, respectively, and the mean duration of pralatrexate treatment was 1.4, 1.8, 2.8, 3.5, and 1.8 months, respectively. Two patients (1 each in the 230 and 270 mg/m^2 cohorts) were still in response and receiving treatment at the time of data cutoff. The maximum number of cycles initiated in the 150, 190, 230, 270, and 325 mg/m^2 cohorts were 2, 7, 15+, 16+, and 4, respectively, and the mean cumulative pralatrexate dose was 596, 710, 1471+, 1646+, and 1194 mg/m^2, respectively.

Seventeen patients had dose reductions because of AEs, of which 14 were due to mucositis (0, 63, 9, 38, and 67% of patients in the 150, 190, 230, 270, and 325 mg/m^2 cohorts, respectively) and 1 each because of fatigue (230 mg/m^2), hand-foot syndrome (270 mg/m^2), and high alanine aminotransferase levels (270 mg/m^2).

Safety

Dose levels and DLTs are summarized for group A in Table 2. Of these patients, one of six patients in the 270 mg/m^2 cohort and two of three patients in the 325 mg/m^2 cohort had DLTs, and the MTD was determined to be 270 mg/m^2. When 10 additional patients were enrolled into the initial MTD cohort (270 mg/m^2), four of these patients had mucositis as a DLT under the original definition for nonhematologic toxicity (i.e., grade 3 mucositis).

After this experience, patients were enrolled into four lower dose cohorts distinguished by dose and duration of infusion. These patients are listed in Table 2 as group B. At the conclusion of the study, 6 of 11 patients treated at 230 mg/m^2 (groups A + B) had grade 2 (four patients) or three (two patients) mucositis, including one patient who had coincident grade 3 neutropenia and thrombocytopenia. Four of eight patients treated at 190 mg/m^2 (groups A + B) had grade 2 (two patients) or 3 (two patients) mucositis. There were no dose-limiting cytopenias at this dose. There was no significant difference in rate of mucositis between patients in group B treated with bolus, versus 1-hour infusion of pralatrexate.

Most patients (97%) experienced at least one AE during the study (Table 3). The most common AEs, grades 1 to 4 and regardless of causality, were mucositis (79%), fatigue (67%), nausea (44%), and epistaxis (28%). Most AEs were grades 1 and 2 in severity. The most common grade 3 and 4 treatment-related AEs by dose ($\leq$190 mg/m^2 and $>$190 mg/m^2) were oral mucositis (33 and 40%) and fatigue (11 and 17%). Patients in the 150 and 190 mg/m^2 cohorts had no grade 3 and 4 hematologic AEs and no grade 4 nonhematologic AEs.

Most patients (95%) experienced at least one AE that the investigator considered related to pralatrexate. The most common treatment-related AEs were oral mucositis (stomatitis; 69%), fatigue (67%), nausea (38%), and epistaxis (26%). Notably, at doses $\leq$190 mg/m^2 there were no treatment-related serious adverse events (SAEs). Six patients (20%) had SAEs at doses more than 190 mg/m^2: five cases of mucositis and one case of headache, all events resolved. One patient died of progressive disease that was not considered related to treatment. No patient had a grade 4 hematologic AE or a grade 4 change in any laboratory value. Grade 3 anemia, grade 3 thrombocytopenia and neutropenia, grade 3 elevation of alanine aminotransferase, and grade 3 hypokalemia occurred in one patient each.

Treatment Response

The overall response rate (ORR) by RECIST was 10% (4/39; 95% confidence interval [CI]: 1–20%), summarized in Table 4. Responses included two patients (5%) with complete response (CR), both of whom were still receiving pralatrexate at the time of this report (time on treatment 26+ and 32+ months, respectively). Of the other two patients (5%) who responded, one patient had a partial response (PR) for 10 months and one patient with PR at the primary site developed leptomeningeal disease at 2 months. The responders were

TABLE 3. Grades 3 and 4 Adverse Events

Grades 3 and 4 Event, *n* (%)	150 mg/m² (*n* = 1)		190 mg/m² (*n* = 8)		230 mg/m² (*n* = 11)		270 mg/m² (*n* = 16)		325 mg/m² (*n* = 3)	
	Gr3	Gr4	Gr3	Gr4	Gr3	Gr4	Gr3	Gr4	Gr3	Gr4
Any grades 3 and 4 event	0	0	4 (50)	0	6 (55)	1 (9)	9 (56)	1 (6)	2 (67)	0
Hematologic										
Anemia	0	0	0	0	1 (9)	0	0	0	0	0
Neutropenia	0	0	0	0	1 (9)	0	0	0	0	0
Thrombocytopenia	0	0	0	0	1 (9)	0	0	0	0	0
Nonhematologic										
Mucositis[a]	0	0	3 (38)	0	2 (18)	1 (9)	6 (38)	0	2 (67)	0
Fatigue	0	0	2 (25)	0	1 (9)	0	4 (25)	0	0	0
Dyspnea	0	0	1 (13)	0	1 (9)	0	0	1 (6)	0	0
Back pain	0	0	0	0	0	0	2 (13)	0	0	0
Dehydration	0	0	0	0	0	0	1 (6)	0	0	0
Headache	0	0	0	0	0	0	1 (6)	0	0	0
Hypoxia	0	0	0	0	1 (9)	0	0	0	0	0
LFT abnormal	0	0	0	0	0	0	1 (6)	0	0	0
Malaise	0	0	0	0	0	0	1 (6)	0	0	0
Noncardiac chest pain	0	0	0	0	0	0	1 (6)	0	0	0
Odynophagia[a]	0	0	0	0	0	0	1 (6)	0	0	0
Pain in extremity	0	0	0	0	1 (9)	0	0	0	0	0
Hand-foot syndrome	0	0	0	0	0	0	1 (6)	0	0	0
Pleural effusion	0	0	0	0	0	0	1 (6)	0	0	0
Pulmonary obstruction	0	0	0	0	0	0	1 (6)	0	0	0
Small intestine obstruction	0	0	0	0	0	0	1 (6)	0	0	0
Somnolence	0	0	0	0	1 (9)	0	0	0	0	0
Syncope	0	0	0	0	1 (9)	0	0	0	0	0

Bolus and infusion cohorts had similar toxicities so were combined for 190 and 230 mg/m² in this table.
[a] Similar terms were grouped (i.e., "stomatitis" and "mucosal inflammation" for mucositis).
LFT, liver function test.

TABLE 4. Treatment Response

Response	Evaluable (*n* = 39)
RECIST response, no. (%)	
CR	2 (5)
PR	2 (5)[a]
SD >2 mo	16 (41)
SD ≤2 mo	10 (26)
PD	9 (23)
Summary rates, no. (%)	
Overall response rate (CR or PR)	4 (10)
Disease control rate (CR, PR, or SD > 2 mo)	20 (51)
Stable disease or better (CR/PR/SD)	30 (77)

[a] One patient with partial response at the primary site developed leptomeningeal disease at 2 mo.
CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

treated at initial doses of 270 mg/m² (CR), 270 mg/m² (PR), 230 mg/m² (CR), and 150 mg/m² (PR), all four had adenocarcinoma, and three had received three or more prior chemotherapy regimens. A waterfall plot summarizing the maximum percent change in tumor size from baseline and dose level for all 39 patients who received pralatrexate is provided (Figure 1).

Twenty-six patients (67%) had best response of stable disease (SD) including 16 (41%) for more than 2 months. The resulting disease control rate (ORR + SD for >2 months) was 51% (20/39; 95% CI: 36–67%), and the rate of SD or better was 77% (30/39; 95% CI: 34–90%) (Table 4).

Pharmacokinetics

The PK profile for racemic pralatrexate is summarized by cohort in Table 5. With both administration schedules, mean values for C_{max} increased dose dependently and were approximately three-fold higher with the 3- to 5-minute bolus than with the 60-minute infusion. With both dosing schedules, mean values for area under the curve (AUC) increased dose dependently up to 230 mg/m² and then appeared to plateau. Other PK parameters, including clearance, volume of distribution, half-life, and fraction excreted in urine, were independent of dose or schedule. The mean plasma concentration-time profile for racemic pralatrexate after the first dose also was similar between dose cohorts (Figure 2).

Potential Predictors of Toxicity

No predictors of toxicity were identified in this study other than dose. There was no correlation between patient experience of mucositis and number of prior chemotherapies, baseline vitamin deficiency as measured by RBC folate, HC, MMA, or

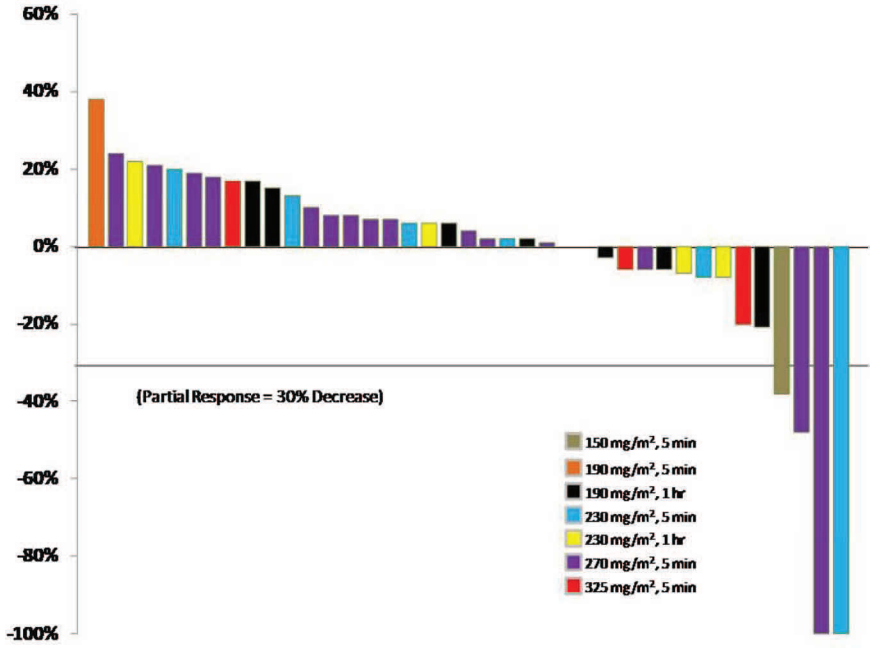

FIGURE 1. Maximum percent change in tumor size from baseline (radiographic only). The dose level of each responder is provided.

TABLE 5. Pharmacokinetic Profile, Racemic Pralatrexate

		Mean Value					
Cohort	***n***	**C_{max} (ng/ml)**	**AUC_∞ (ng/ml ·min)**	**CL_{tot} (ml/min)**	**Vd_{ss} (liter)**	**$t_{1/2}$ (min)**	**f_e (%)**
Overall	37	52,712	1,832,138	361	75	1280	29
150 mg/m^2 bolus	1	24,729	551,967	601	356	4080	24
190 mg/m^2 bolus	3	28,141	1,290,391	351	63	869	20
230 mg/m^2 bolus	4[a]	55,931	2,082,075	242	63	1941	35
270 mg/m^2 bolus	16	64,050	1,792,341	329	52	1187	28
325 mg/m^2 bolus	3	66,155	2,167,199	474	182	2699	30
190 mg/m^2 infusion	5	10,771	1,200,685	489	67	485	32
230 mg/m^2 infusion	5	17,192	1,853,588	346	57	545	34

[a] One outlier was removed for C_{max} and AUC calculation.

C_{max}, maximum concentration; AUC_∞, area under the concentration-time curve from time 0 to infinity; CL_{tot}, total clearance; Vd_{ss}, volume of distribution at steady state; $t_{1/2}$, terminal half-life; f_e, fraction excreted in urine.

PK parameters (C_{max} or AUC) within each dose level. Vitamin B12 and folic acid supplementation initiated at least 7 days before initiation of pralatrexate improved vitamin status in patients. RBC folate increased from a median of 738 to 764 ng/ml, MMA decreased from a median of 186 to 164 nmol/liter, and HC decreased from a median of 10.5 to 9.1 μmol/liter before the first dose of pralatrexate. Although the MTD of pralatrexate was found to be higher in this study with the use of vitamin supplementation, the overall rate of mucositis (any grade) was similar in patients treated at doses $\leq$190 mg/m^2 and $>$190 mg/m^2. High-grade mucositis resulting in a serious AE was more likely, and there was a higher rate of treatment-related SAEs and myelosuppression at doses more than 190 mg/m^2.

DISCUSSION

Biweekly administration of pralatrexate with vitamin supplementation was safe in patients with previously treated, advanced NSCLC. As in the previous studies of pralatrexate without vitamin supplementation,[7–9] oral mucositis remained the most common DLT. Other AEs were consistent with those in previous studies of pralatrexate in NSCLC, and most events were mild and reversible. There were no treatment-related serious AEs, grade 3 and 4 hematologic AEs, or grade 4 nonhematologic AEs recorded in patients treated at doses $\leq$190 mg/m^2. Rates of any grade and grades 3 and 4 mucositis were similar at doses $>$190 mg/m^2 and $\leq$190 mg/m^2.

Results from the safety analysis indicated 190 mg/m^2, along with vitamin supplementation, to be the optimal starting dose. This dose is higher than the MTD in the prior phase 1 and phase 2 studies that did not include vitamin supplementation (170 and 135 mg/m^2 q2w, respectively).[6,7] With pralatrexate delivered at a dose of 190 mg/m^2 IV q2w, mucositis remains the DLT, and this study failed to demonstrate that vitamin supplementation prevents mucositis. Be-

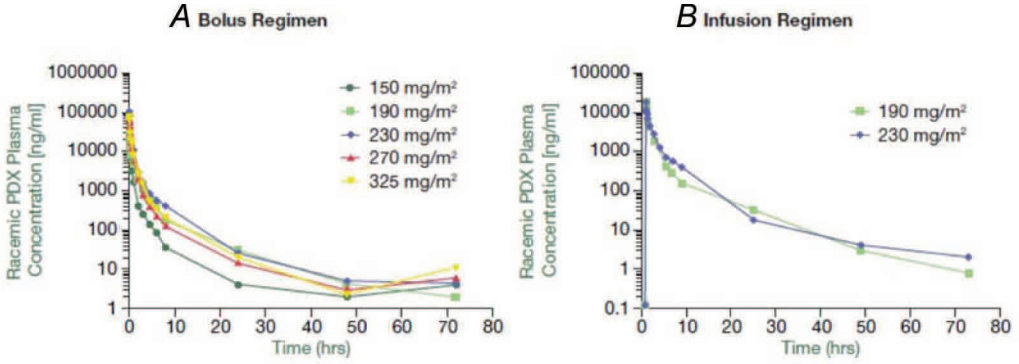

FIGURE 2. *A* and *B*, Mean plasma concentration-time profile on day 1 (0–80 hours), racemic pralatrexate.

fore publication of this phase 1 study, the results of a phase 2 study which delivered pralatrexate plus vitamins at a median dose of 190 mg/m^2 to 100 patients with previously-treated metastatic NSCLC were reported in an abstract. In the phase 2 study, rates of mucositis were 34% grade 2, 20% grade 3, and 3% grade 4, and 21% of patients discontinued pralatrexate because of mucositis, the majority of which occurred during cycle 1.[13]

In this phase 1 study, the additional toxicities encountered during planned expansion of the cohort at the initial MTD (270 mg/m^2) highlight the importance of cohort expansion in phase 1 trials to account for patient heterogeneity. One could argue that smaller dose increments might have allowed discovery of intermediate dose levels with less toxicity. However, in this study, as in prior phase 1 and 2 studies of pralatrexate, no clinical predictive factors for the development or severity of mucositis were identified. Until such discoveries are made, future pralatrexate clinical trials should include individualized treatment modification and prospective mucositis management strategies to maximize pralatrexate exposure and maintain tolerability.

The PK evaluations in this phase 1 study were consistent with those reported previously without vitamin supplementation in patients with NSCLC,[7] suggesting that the addition of vitamin supplementation did not significantly alter the PK profile. Within this study, the PK profiles of bolus and 1-hour infusion dosing were similar, aside from expected differences in C_{max}. The AUC of pralatrexate increased dose dependently with both administration schedules but appeared to reach a plateau at 230 mg/m^2. There was no significant difference in rates of mucositis when pralatrexate was administered as a bolus versus 1-hour infusion.

Pralatrexate therapy was associated with durable CR in two patients (26+ and 32+ months) and PR in two additional patients (10 and 2 months) for an ORR of 10%. Several other patients had SD, including prolonged SD for more than 2 months in 41% and ≤2 months in 26%, thus resulting in disease control in approximately one half of patients (51%) and SD or better in most patients (77%). Of note, duration of response in the two patients with recurrent NSCLC treated with pralatrexate (26+ and 32+ months) is longer than the median survival (≤8 months) of patients in this population.[14] Long duration of response was also observed with pralatrexate in relapsed/refractory peripheral T-cell lymphoma.[5]

Previous studies reported objective treatment responses with single-agent pralatrexate at doses of 150 to 170 mg/m^2 q2w[7,8] or the combination of a taxane plus pralatrexate 60 to 110 mg/m^2 q2w (without vitamin supplementation) or 80 to 140 mg/m^2 (with vitamin supplementation).[9] In this trial, clinical activity was evident at doses of 150 mg/m^2 and higher.

Because of a small number of tissue samples, meaningful correlation between biomarker results and safety or treatment response could not be evaluated. FFPE tumor tissue was available in sufficient quantity for gene expression analysis in only six patients, and most samples represented primary tumor removed at surgery and not contemporary with chemotherapy for metastatic disease.

In this study, all of the patients observed to have major radiologic responses had adenocarcinoma histology. Similarly, in the phase 2 study of pralatrexate recently reported, there were trends to greater efficacy with pralatrexate in patients with nonsquamous histology and (not surprisingly) in patients who did not stop taking pralatrexate on account of mucositis.[13] Another antifolate drug for NSCLC, pemetrexed, has also been observed to be more effective in patients with nonsquamous histology.[15] Pralatrexate targets primarily DHFR, whereas pemetrexed targets primarily TS. The biological mechanisms behind these parallel observations have yet to be elucidated.

In conclusion, pralatrexate in combination with vitamin B_{12} and folic acid was safe in patients with previously treated NSCLC, and durable responses were observed. Oral mucositis remained the DLT, but higher doses than previous trials were tolerated most likely because of vitamin supplementation. Observations in this study, and other phase 2 studies in NSCLC, show trends for improved efficacy in nonsquamous histology. However, molecular markers for selection of patients more likely to benefit from pralatrexate have yet to be elucidated. Future pralatrexate clinical trials should include individualized treatment modification and prospective mucositis management strategies to maximize pralatrexate exposure and maintain tolerability.

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